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CITATION:

Minezawa, Mitsuru ...[et al]. Cytogenetics of Bolivian Endemic Red Howler Monkeys (Alouatta seniculus sara): Accessory chromosomes and Y-autosome translocation related numerical variations. Kyoto University overseas research reports of new world monkeys 1986, 5: 7-16

ISSUE DATE:

1986

URL:

http://hdl.handle.net/2433/199627

Kyoto University Overseas Research
Reports of New World Monkeys (1985) 5: 7–16
Kyoto University Primate Research Institute

Cytogenetics of Bolivian Endemic Red Howler Monkeys (*Alouatta seniculus sara*): Accessory chromosomes and Y-autosome translocation related numerical variations

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ABSTRACT

A karyotypic study on red howler monkeys, *Alouatta seniculus sara*, captured in Santa Cruz, Bolivia was carried out. The range of chromosome number was $2n = 48$ to 51. Female had 9 pairs bi-arm chromosomes (including the submetacentric X-chromosome), male, however, had 7 pairs and two unpaied bi-arm chromosomes. The number of acrocentric chromosome (excluding the two smallest pairs, B_1 and B_2, because of their numerical variability) was 26 in female and 27 in male. A different chromosome number was frequently observed among cells even in a same individual. A G-band karyotype reveals a male specific acrocentric chromosome can be derived from the normal 6th chromosome by centromeric fission and insertion of an additional segment (probably Y-chromosome) between the telomeric end of two arms. The mnuerical range of B_1 was 2 to 4 and that of B_2 was 2 to 6 and that of B_1+B_2 was 5 to 8. The B_2 chromosome is numerically more variable than the B_1, and male has more B_1 chromosome (2.88) than female (2.13). Probably the facts, B_2 is C-band negative and B_1 is centromeric C-band positive, might affect chromosome movement at meiotic and mitotic division.

The karyotype of Bolivian monkeys so far studied was highly endemic. The distribution of endemic species overlapped a paleoecological forest refuge of this region suggested by Brown (1982).

INTRODUCTION

Six species of howler monkeys (*Alouatta palliatta*, *A. blezebul*, *A. seniculus*, *A. caraya*, *A. fusca*, and *A. villosa* or *pigra*) distributed widely from Central America to Northern Argentine of South America (Hill, 1962; Naipier, 1976; Mittermeier and Coimbra-Filho, 1981; Wolfheim, 1983). The existence three species of howler monkeys (*A. seniculus*, *A. caraya*, and *A. fusca*) were reported in Bolivian lowland (Hill, 1962). A distribution of two species (*A. seniculus* and *A. caraya*) in Bolivia was confirmed (Naipier, 1976; Wolfheim, 1983). *Alouatta*

Table 1 Chromosome studies in *Alouatta*.

Species	2n	Chromosomes N—A[1]	A[2]	X	Y	Authors
A. palliata	53, 54	22	30	SM	Tr[3]	Ma et al. (1975)
A. fusca	50, 49	20	28, 27	SM	A	Koiffmann & Saldanha (1974)
A. seniculus	43, 44	12, 13	28, 29	A	SM	Yunis et al. (1976)
	45	14	30, 31			
A. caraya	52	20	30	SM	A	De Boer (1974)

[1] Non-acrocentric,; [2] Acrocentric; [3] Translocated to autosome

fusca or *A. beniensis* was also reported in Beni, Bolivia by Lönnberg (1941). *A. fusca*, however, normally distributed in eastern coast of Brazil, a thousand kilometers apart from Beni, Bolivia. Therefore, Mittermeier and Coimbra-Filho (1981) doubted its existence in Bolivia.

A red howler monkey, *A. seniculus*, has the widest habitat area of all howler species and has many subspecies (Wolfheim, 1983). That of Central Bolivia is classified as *A. s. sara* which at first was classified as *A. sara* by Elliot (1910).

At present, the karyotypes of 4 species (*A. palliatta*, A. *fusca*, *A. caraya* and *A. seniculus*) were investigated and have rather large difference among them (Table 1), which is comparable to that of *Aotus* (Ma, 1981). We present here a result of karyotypic study on Bolivian red howler monkeys captured in Santa Cruz, Bolivia and show their inter- and intraindividual numerical variations of karyotypes by a Y-autosome translocation and two types of accessory chromosomes.

MATERIALS AND METHODS

Peripheral blood samples were taken from 33 red howler monkeys (*Alouatta seniculus sara*), including 20 females and 11 males, belonging to 7 groups. These animals captured in a forest

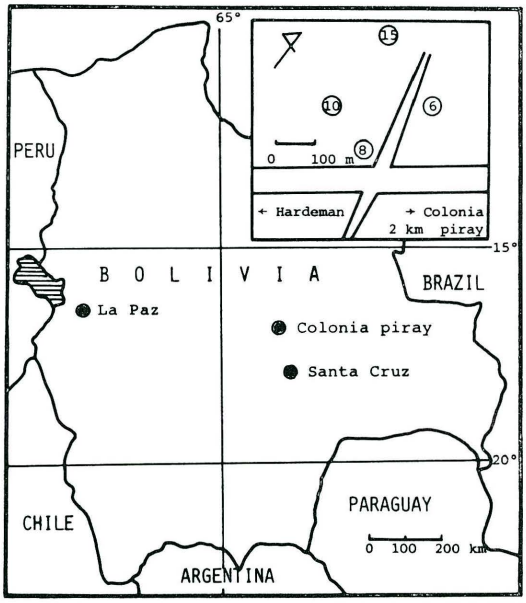

Fig. 1. Study area in central Bolivia and location of groups near Colonia Piray.

Table 2 Central Bolivian red howler monkeys, *Alouatta seniculus sara*, captured near Colonia Piray.

Group	Total	Female	Male
6	7	3	4
8	5	3	2
9	1		1
10	5	3	2
13	3	2	1
15	8	5	3
16	4	4	
Total	33	20	13

near the Colonia Piray, 100 km north to Santa Cruz de la Sierra, Bolivia (Fig. 1, Table 2).

The whole blood samples were cultured 72 hours with RPMI-1640 (containing PHA-M, 10% FCS and antibiotics) within 5 days of collections at Instituto Bioclinico Central, Santa Cruz, and cells were fixed by the standard methods. The fixed samples were carried back to the Primate Research Institute, Kyoto University. The metaphase chromosome was sequentially stained for standard Giemsa, and G- and C-band with ASG (Sumner et al., 1971) and BSG (Sumner, 1972) techniques.

A minimum of 10 metaphase from each specimen was analysed under the microscope for counting the chromosome number and gross morphology. If found two or more cells with same complements which are different from modal karyotype, 50 more cells were observed to score each karyotype.

RESULTS

The chromosome numbers among Central Bolivian red howler monkeys (*Alouatta seniculus sara*) were highly variable and their range was $2n = 48$ to 51 (Table 3). A different chromosome number was frequently observed among cells even in a same individual. The numerical variations of chromosome number were due to two factors: 1. The smallest two chromosome pairs (B_1 and B_2) numerically differ individual to individual and also among cells of a same individual, 2. The numbers of bi-arm chromosomes (including the X-chromosome) differ between male and female. Female had 9 pairs bi-arm chromosomes, male, however, had seven pairs and two unpaired biarmed chromosomes. The numbers of acrocentric chromosomes were 26 in female and 27 in male.

A G-band karyotype (Fig. 2) reveals one of unpaired bi-arm chromosomes in male is X-chromosome and another is a chromosome of the 6th pair. Male also have a unique unpaired

Table 3 Karyotypes of Bolivian howler monkeys, *Aoluatta seniculus sara*.

	Nos. Anim.	Average modal chromosome number (S.D.)				
		Total	Bi-arm	B_1	B_2	B_1+B_2
All animals	33	50.061 (0.659)	16.970 (1.015)	2.515 (0.566)	4.061 (0.747)	6.576 (0.830)
Animals with one karyotype	22	50.091 (0.750)	17 (1.023)	2.5 (0.598)	4.091 (0.868)	6.591 (0.908)
Animals with multi-karyotype	11	50 (0.447)	16.909 (1.045)	2.545 (0.522)	4 (0.447)	6.545 (0.688)
Female	16	50.063 (0.443)	18 (0)	2.125 (0.342)	3.938 (0.574)	6.063 (0.443)
Male	17	50.598 (0.827)	16 (0)	2.882 (0.485)	4.176 (0.883)	7.059 (0.827)

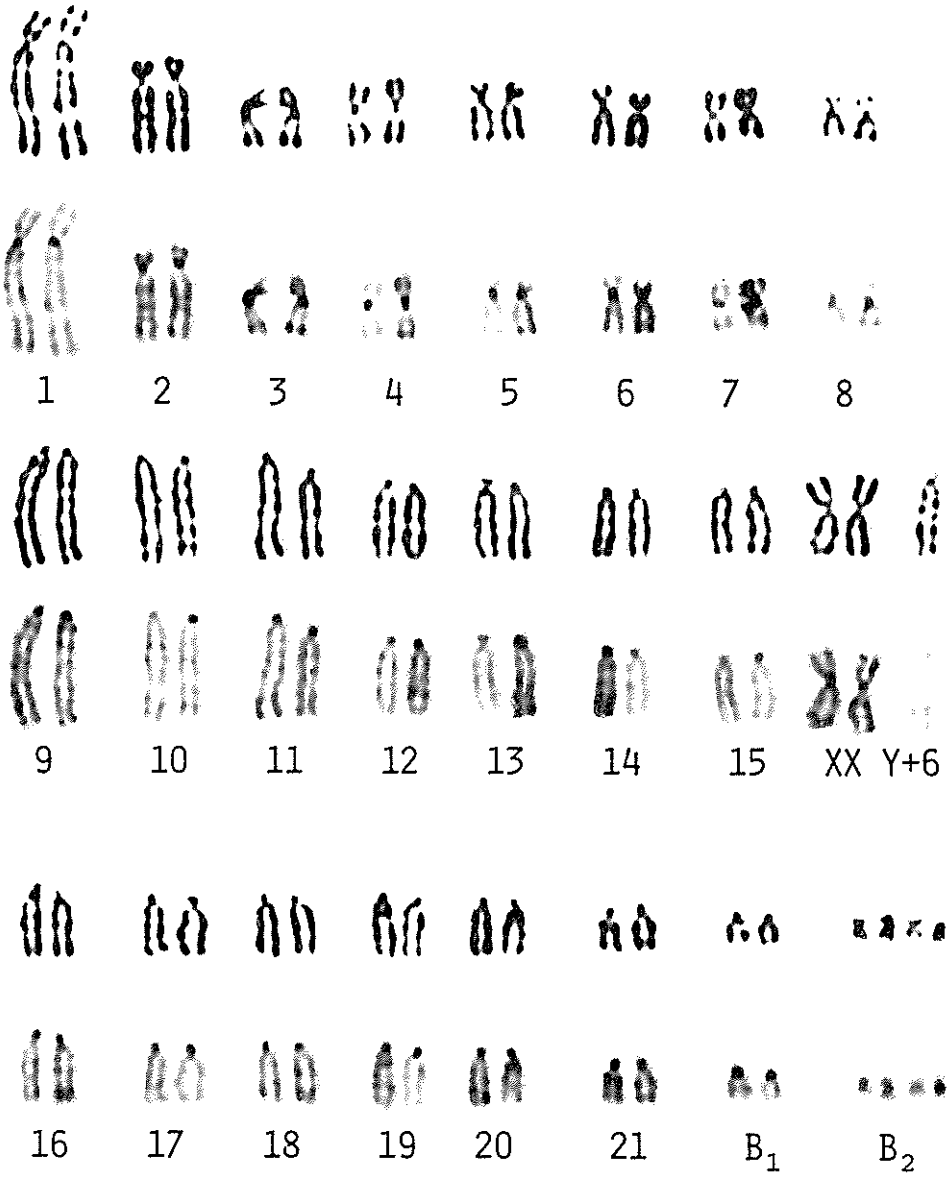

Fig. 2. G- and C-band karyotype of female *Alouatta seniculus sara*, and unique acrocentric chromosome of male (Y+6).

acrocentric chromosome which has similar size to 11th pair. When compared G-band of the 6th chromosome with unpaired acrocentric one, as shown Figure 3, male specific acrocentric chromosome can be derived from the normal 6th chromosome by centromeric fission and insertion of an additional segment between the telomeric end of two arms. Possibly this additional segment is a translocated Y-chromosome.

C-bands were positively stained centromeric region of all chromosomes except for a B_2 chromosome. No telomeric nor interstitial C-band was observed in any chromosome.

Intraindividual variation of chromosome number was found in 11 animals, 6 females and

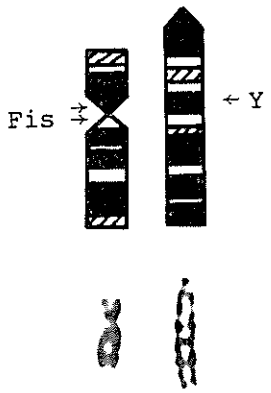

Fig. 3. Derivation of the male specific acrocentric chromosome from the 6th chromosome by centric fission and translocation of Y segment between telomeric end of both arms.

5 males. The B_1 differed numerically among cells of same individual and the B_2 differed in 8 and both of the two smallest differed in 2 animals. The modal numbers of B_1 and B_2 between animals with uni- and multi-karyotype were not significantly different. The modal numbers were used to calculate average numbers of the small chromosomes. The average of chromosome number of B_1 and B_2 was 2.52 and 4.06, respectively. The numerical range of B_1 was 2 to 4 and that of B_2 was 2 to 6 and that of B_1+B_2 was 5 to 8. As shown above, the B_2 chromosome is numerically more variable than the B_1. When compared female to male, male has more B_1 chromosome (2.88) than female (2.13) ($p<0.001$). B_2, however, shows no significant difference between both sexes.

A comparison of the number of the smallest two chromosome pairs was tried among the groups which consisted of more than five individuals observed. Because the numbers of B_1 chromosome show the difference between two sexes, only B_2 chromosome could reach sufficient individual number and be compared among the groups. Only four groups (G-6, G-8, G-10 and G-15) fulfilled this number and a comparison was carried out among them. The average numbers of B_2 chromosome of G-6, G-8, G-10 and G-15 were 4.29, 4.80, 4.00 and 3.88, respectively. Though numerical differences of B_2 between G-6 and G-15, and between G-8 and G-15 were not significant, fairly large differences ($0.1<p<0.05$) were demonstrated.

DISCUSSION

The karyotype of the red howler monkeys of northern Santa Cruz, Bolivia differs from that of other howler species so far studied. Strangely, intraspecific karyotypic difference of *A. seniculus* is larger than differences among congeneric species studied (Table 1). By G-band comparison between *A. seniculus* and *A. palliatta*, tentatively 18 homologous pairs (including 2 inversion and a translocation) are recognized, but the 4th, 9th, 18th and 19th pairs of Bolivian karyotype can not find their homologues in a Panamanian species (*A. palliatta*) karyotype. Though *A. palliatta* has translocated Y-chromosome to autosome as *A. seniculus sara*, the recipient autosomes differ each other (Ma et al., 1975).

The numerical difference of the smallest two chromosome pairs (B_1 and B_2) did not affect morphological characters of Bolivian red howler monkeys. Therefore, these two pairs seem

to be genetically inactive and the existence of the numerical variations is not an aneuploidy but accessory chromosomes (or B-chromosome or supernumerary chromosome) by its definitions (Munzting, 1974). Intraindividual karyotypic variations are well known in chimeric dizygotic twins of callitrichids (Gngozian, 1969). Howler monkeys, however, are usually signle born and the frequency of individuals with multikaryotypes is high, therefore the chimerism between dizygotic twins like callitrichids does not fit in the case of Bolivian red howler monkeys. An accessory chromosomes were also recognized in northern Colombian red howler monkeys (3 to 5/individual) (Yunis et al., 1976). Their accessory chromosomes are only one type and C-band negative and smaller than those of Bolivian subspecies. *A. fusca* in eastern coast of Brazil showed an numerical variation of karyotype by a different number of the smallest pair. This smallest pair of Brazilian howler monkey seems to be accessory chromosome, but was mentioned as aneuploidy by Koiffmann and Saldanha (1974). As mentioned above, accessory chromosomes and Y-autosome translocation might be occur several times and not be unique event among howler monkey species.

Of the two types accessory chromosomes, B_2 is C-band negative and B_1 is centromeric C-band positive. This fact probably means B_2 has a mal-functional kinetocore and affects the dynamics of two accessory chromosomes. The B_2 shows abnormal effect on meiotic and mitotic chromosome movement deffect in the B_2 may have mal-functional effect on both mitotic and meiotic chromosome movements. Therefore, a chromosomal non disjunction may occur easily on B_2, and the B_2 may reveal greater variability than the B_1. Several grasshopper species showed sex-chromosome related accessory chromosome movements (White 1973). If sex-chromosome related movement occurred in meiotic division of male red howler monkey, the different accessory chromosome number between both sex can be explained. If B_1 chromosomes have tendency to move opposite side of X-chromosome in male meiotic divisions, the gamates without X-chromosome shall have more B_1 chromosomes than those with X-chromosome and as a results male shall have more B_1 chromosomes than female.

In this study area, a fairly large but not significant inter group difference of the accessory chromosome number was observed. Considering the large congeneric and conspecific karyotypic differences among howler monkeys, a intergroup difference may suggest a diversifying tendency of this taxa.

An Y-autosome translocation was observed in many mammalian species. In primate species, especially in New World monkeys this event occurred frequently, ex. *Callimico* goeldii (De Boer 1974), *Cacajao rubicundus* (Koiffmann and Saldanha, 1981), *Aotus* sp. (Ma et al., 1976) and *Alouatta* sp. (Ma et al., 1975). On the other hand in Old World monkeys only *Presbytes* sp. showed a Y-autosome translocation (Dutrillaux et al., 1984).

Craniometrical study of red howler monkeys by Watanabe (1982) recognized existence of a cline and demonstrated that northern Bolivian race is on the clinal line. On the other hand, central Bolivian red howler monkeys were classified as *A. sara* by Elliot (1910) and their karyotypes are largely different from those of northero Colombian race, *A. seniculus seniculus*, (more than 10 chromosomal rearrangements are required to reconstruct from one karyotype to another). Therefore, central Bolivian red howler monkeys should be splitted from *A. seniculus* as a independent species, if admitted splitting *Aotus* and *Salmiri* from one species to many species mainly by karyotypic differentiation (Hershkovitz, 1983, 1984). Because the karyotypic variability of genus *Alouatta* is comparable to that of *Aotus*, further cytogenetic studies of *A. seniculus* shall discover more unique and habitat specific karyotypes.

A distribution of *A. seniculus sara* is not throughly investigated but is apparently isolated

in environs of Rio Piray, right bank tributary of the Rio Yapacani, Santa Cruz, Bolivia (Napier 1976). Other central Bolivian primates also reveal high endemicity. Apart from the ploblematic existence of *A. fusca* or *A. beniensis*, 8 genera, 8 species of primates were reported from central Bolivian region (Wolfheim, 1983). Three of the central Bolivian monkey species or subspecies, belonging to *Aotus*, *Callicebus* and *Alouatta*, possess endemic karyotypes clearly differentiated from those of the congenerics or conspecifics inhabitated in other regions (Hershkovitz, 1983, Minezawa and Valdivia 1984a). And Hershkovitz (1984) recognized Bolivian squirrel monkey, *Saimiri boliviensis*, as a independent species based on mainly a karyotype and a coat color pattern. Moreover, a central Bolivian tufted capuchin, *Cebus apella*, shows several unique chromosomal variations when compared with Brazilian conspecific animals (Freitas and Seuanez, 1982; Minezawa and Valdivia, 1984b) and is on the course of speciation. A black tailed marmoset, *Callithrix argentata meranura*, could be thought as endemic subspecies of this region by its habitat distribution (Hershkovitz, 1977; Wolfheim 1983). The other two central Bolivian monkeys, *Saguinus fuscicollis weddeli* and *Ateles paniscus*, were not extensively studied but their distributional features apparently suggested that they originated from more northern region (Wolfheim, 1983). As discussed above, so far 6 species of 8 central Bolivian primates are somehow endemic, though the degrees of endemicities of these monkeys vary from species to species.

A mapping of distributions of the central Bolivian monkeys on the same sheet (Hershkovitz, 1963, 1977, 1983, 1984; Wolfheim, 1983), demonstrates all species overlapped the almost same region where *A. seniculus sara* were inhabitated (Napier, 1976) (Fig. 4). This exact region almost overlaps a paleoecological forest refuge of this region (called Beni or Yungus 2) suggested by Brown (1982) based on a distribution of neotropical forest butterflies. Besides this, a distribution of neotropical forest plant, *Cariniana esterllensis* also overlaps to this refugia center (Prance, 1981). Apart from this phenomenon, Kinzey (1982) discussed a relationship between neotropical primate distribution and Pleistocene forest refuges. He suggested that forest refuges play an important role on formation of pretent-day primate distribution. Kinzey (1982) also suggested central Bolivian dusky titi, *Callicebus moloch donacophilus*, originated in Guapore refugia. Figure 4 reveals stronger geographical relation between the distribution of *Callicebus moloch donacophilus* and Beni refugia and also suggests *Callithrix argentata melanura* originated in Guapore refugia.

Fig. 4. Geographical distributions of the Bolivian endemic primates species and subspecies (see text). All species overlapped in blacked area.

On the other hand, Hershkovitz (1963, 1972) suggested that present *Callicebus* and callitrichids have evolved under the contribution of major Amazonian tributaries as barriers. Kinzey (1982), however, disagreed "centripetal dispersal" view of Hershkovitz (1963) and offered considerable evidence which is not consistent with the river barrier hypothesis. From the viewpoint of central Bolivian biota where the endemic centers of primates, insects, and plants are overlapped, Bolivian primate fauna might have been formed under a circumstance of Quaternary refugia. Thereafter, following a recovery of forest, each endemic species variously expanded its area depending on a niche of each species and an interaction among environmental factors, such as Amazonian tributaries and other refuges.

Highly diversified and area specific karyotypes of *Aotus* (Hershkovitz, 1983) were well known and the range of karyotypic variation of *Alouatta* and *Callicebus* (Minezawa and Valdivia, 1984a) was comparable to that of *Aotus*. If Pleistocene refuges played a major role on evolutionary process of neotropical primates and if we investigated cytogenetically these species as extensively as *Aotus*, we could expect many other karyotypically distinct species or subspecies with limited distribution, which could be highly correlated to the distribution of chromosomal races of *Aotus*, in *Alouatta* and *Callicebus*.

ACKNOWLEDGEMENTS

This research was supported by the overseas Scientific Research Funds of the Ministry Education, Science and Culture, Japan, and constituted a part of the research project of the Primate Research Institute, Kyoto University headed by Dr. Y. Nogami. We would like to express our sincere thanks to Prof. N. Kempff Mercado, el miembro de la Academia Ciencias de Bolivia. We also wish to thanks to Dr. Demetrio Pessoa R. and the other members of the Instituto Bioclinico Central, Santa Crus, Bolivia, for their kind collaboration in this work. We are grateful to Mr. Jiro Ohnishi, Zoological Garden Supplier, for his helpful collaboration in obtaining samples.

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